TESTING DESIGN

Question 1: Aquatic and terrestrial "Level" assessment scheme

Ted Kuchnicki, Ph.D. (Pest Management Regulatory Agency, Health Canada)

Four assessment levels are proposed for determining chemical risk to non-target plants. The levels progress from a deterministic assessment (Level 1) to more refined assessments (Levels 2-4). Groups of species identified as being at risk at one level will trigger assessment at higher levels. There are various options for refining aquatic and terrestrial plant assessments. These include a more realistic exposure estimation, additional species testing (to improve characterization of sensitivity), or examination of the ratio of an exposure distribution over a sensitivity distribution (rather than a ratio of single points). These refinements are intended to reduce the level of uncertainty in an assessment.

- **S** What are the advantages and disadvantages of the proposed tiered approach for assessing chemical risk to plants?
- **S** What refinements to plant assessment are recommended at each level?
- **S** What are the Panel's suggestions for an improved or more detailed plant assessment scheme beyond that already discussed?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

Question 2: Uncertainty factors

Michael Davy, B.S. (Office of Pesticide Programs, EPA)

Uncertainty factors can be used in estimating the risk of chemicals to aquatic and terrestrial plants. Uncertainty factors have been defined in a number of ways, but most are related to two concepts: 1) addressing uncertainty due to variability in testing and extrapolation to untested species, and 2) addressing uncertainty due to the limited data. The agencies have proposed expansion of test species to address these issues; however, uncertainty factors may also improve confidence in risk assessments.

- **S** What are the advantages and disadvantages of using uncertainty factors in lieu of additional aquatic or terrestrial plant toxicity tests? How should they be applied?
- **S** What are the Panel's thoughts on the applicability of aquatic plant uncertainty factors (as currently used by the OPPT) to terrestrial plants? Are entirely different uncertainty factors needed?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

ENDPOINTS AND TRIGGERS

Question 3: Aquatic triggers for progression to higher levels

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

Effective concentration (EC) values are used to determine chemical risk to aquatic plants. Population endpoints (e.g., biomass as measured in algae) are measured over a full life cycle, whereas individual endpoints (e.g., plant length as measured in *Myriophyllum* spp.) can be measured partially or over a full life cycle. Various options have been presented for selection of EC values.

- **S** What aquatic EC values (NOAEC, EC_{50}, or other) are appropriate for this testing scheme and for calculating risk quotients at Level 1?

- **S** What aquatic EC values (e.g., EC_{10}, EC_{25}, etc.) should be used for population and individual plant parameters?

Lead Discussants: (selected aquatic Panel members)

Question 4: Terrestrial triggers for progression to higher levels

Michael Davy, B.S. (Office of Pesticide Programs, EPA)

Progression through the level system is influenced by the sensitivity of the plant and exposure to the test chemical. Detection of this sensitivity depends on the endpoints selected, as well as when they are measured during the plant life cycle. In current assessments, individual plant parameters (e.g., phytotoxicity, height, dry weight) are measured for the determination of chemical risk to non-target terrestrial plants. Currently, population effects are not predicted but the EPA and PMRA expect to address these impacts in the near future. At the present time, the EC_{25} value for the most sensitive parameter is used in the calculation of a risk quotient at Level 1.

- **S** What parameters (height, dry weight, survival, etc.) are appropriate to measure at Level 1? Which EC value should be used for each parameter?

- **S** What are the advantages and disadvantages of each parameter with respect to the expense and time involved in conducting the test and the ability of the test results to accurately predict effects?

- **S** How can individual plant parameters be used to predict population effects?

Lead Discussants: (selected terrestrial Panel members)

Question 5: Consideration of physiological and biochemical markers

Michael Davy, B.S. (Office of Pesticide Programs, EPA)

In addition to established measurement endpoints (gross acute), recent literature suggests it is important to consider alternative endpoints pertaining to physiological and biochemical effects (e.g., O_2 production, carbon fixation, etc.). The endpoints assessed in seedling emergence and vegetative vigor tests may not fully detect physiological changes that are detrimental to plants.

- **S** How important is it to consider physiological or biochemical endpoints when determining chemical risk to plants?

- **S** What physiological or biochemical changes would be considered adverse? Please quantify (e.g., 20%, 50%, etc.).

- **S** What would be the advantages and disadvantages of replacing seedling emergence and vegetative vigor endpoints with physiological and biochemical endpoints?

- **S** Which additional endpoints could be used to assess hazard to non-target plants?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

Question 6: Aquatic and terrestrial reproductive effects

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

Because existing plant toxicity tests do not provide an adequate trigger for reproductive testing, the PMRA and the EPA propose that reproductive testing be conducted at Level 1. For aquatic plant assessments, we propose the testing of two species (rice and nodding smartweed). For terrestrial plant assessments, we propose two of three test species [cherry, mouse-ear cress (*Arabidopsis thaliana*), and canola (*Brassica rapa*)]. The agencies request guidance on how to refine the assessment of reproductively sensitive plant species.

- **S** What are the advantages and disadvantages of the proposal to assess reproductive effects at Level 1 versus higher levels?

- **S** What are the advantages and disadvantages of partial- versus full-life cycle testing?

- **S** For full life-cycle testing, what are the advantages and disadvantages of testing mouse-ear cress versus canola?

- **S** What are some alternative approaches and species that address potential reproductive effects?

- **S** What reproductive testing endpoints (e.g., pollen viability, seed formation, etc.) are critical for Level progression and for assessing chemical risk to non-target plants?

- **S** What reproductive EC values (e.g., NOAEC, EC_{05}) are critical for Level progression and for assessing the risk to non-target plants?

- **S** How critical is it to consider both modes of reproduction (sexual versus asexual)?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

AQUATIC PLANT TESTING

Question 7: Marine algal toxicity testing

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

The range of response among different marine algae can be as great as is observed in freshwater species. Currently, only one marine algal species test is required at Level 1. Researchers have recommended a test battery which includes species of diatoms, green algae, and dinoflagellates as well as golden-brown algae. This test battery should provide a range of responses until sufficient comparative toxicity studies are available to determine sensitivity ratios. In addition to the currently required marine diatom, the proposed plant testing scheme requires the testing of one marine algal species in each of three previously unrepresented Divisions (Chyrsophycophyta, Pyrrhophycophyta, and Rhodophyta).

- **S** Are the four proposed marine algal species representative? What other marine algal species should be considered? Why?

- **S** What are the limitations of the protocols and the availability of the proposed marine algal species?

Lead Discussants: (selected aquatic Panel members)

Question 8: Aerial exposure testing of floating aquatic plants

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

Chemical exposure can occur from drift deposited onto the leaf surfaces of floating plants. For contact toxicants, it is not sufficient to test only aquatic exposure (i.e., chemical dissolved in water). It has been observed that the sensitivity of *Lemna* to a contact herbicide can increase several-fold with a foliar exposure compared to the conventional exposure through the growth medium. Although there are limited data on aerial exposure, both Agencies believe that this type of study should be conducted on a routine basis.

- **S** What are the pros and cons of routinely requiring foliar exposure tests for floating aquatic plants?

- **S** How can the methodology by Lockhart *et al.* (1989) be modified for future testing requirements?

- **S** What research needs can be identified for foliar exposure testing?

Lead Discussants: (selected aquatic Panel members)

Question 9: Submersed aquatic vascular plant testing

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

Submersed aquatic vascular plants are morphologically different compared to terrestrial vascular plants or algae. Currently, *Lemna* spp. (floating) are used to predict effects on submersed aquatic vascular plants. As *Lemna* is a monocotyledon, it would be preferable to also require a representative dicotyledon. The agencies recommend *Myriophyllum* spp. to represent dicotyledonous submersed aquatic species.

- **S** What is the SAP's opinion on the proposed toxicity test requirement with a submersed aquatic vascular species?

- **S** What are the Panel's thoughts on the selection of *Myriophyllum* spp. to represent dicotyledonous submersed aquatic vascular plants?

- **S** What other monocotyledonous and dicotyledonous submersed species should be considered? Why?

Lead Discussants: (selected aquatic Panel members)

Question 10: Emergent aquatic vascular plant testing

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

Currently, *Lemna* spp. (floating) and terrestrial vascular plants are used to predict the effects of toxicants on emergent aquatic vascular plants. However, there are no available data to support the use of these as surrogates for emergent aquatic plants. Many emergent aquatic plant species prosper in both aquatic and terrestrial habitats. In an aquatic exposure scenario, emergents are unique in that they can be exposed *via* root or stem uptake of contaminated water in addition to foliar exposure from an over-spray.

- **S** What are the Panel's thoughts regarding the physiological differences between emergent aquatic and terrestrial vascular species or *Lemna* sp. to support the proposed approach?

- **S** What are the Panel's thoughts on the selection of rice and nodding smartweed to represent emergent aquatic vascular plants?

- **S** What other emergent aquatic vascular plant species can the Panel recommend to satisfy both terrestrial and aquatic testing requirements?

- **S** How important is it to consider routes of exposure other than foliar exposure to emergent aquatic species (i.e., absorption from the water column by the submersed stem or from sediments *via* the roots)?

- **S** What is the Panel's opinion regarding extrapolation from terrestrial vascular species or *Lemna* spp. to emergent rooted aquatic vascular plants?

Lead Discussants: (selected aquatic Panel members)

SCIENTIFIC RATIONALE FOR TESTING WAIVERS

Question 11: Mode of action

Michael Davy, B.S. (Office of Pesticide Programs, EPA)

The agencies are proposing a seedling emergence and a vegetative vigor test at Level 1. Various factors contribute to eliciting toxicity of a particular chemical (e.g., mechanism of uptake by a plant, the mode of action of the chemical within a plant, application parameters, etc.). Information on these factors could reduce the need to conduct a seedling emergence or vegetative vigor test.

- **S** What criteria might we use for data waivers based on the mode of uptake and the mode of action of a chemical?

- **S** How could the number and types of tests be reduced with respect to application parameters (i.e., timing, method, etc.)?

- What other factors might reduce the amount of testing required?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

TERRESTRIAL PLANT TESTING

Question 12: Terrestrial Species

Michael Davy, B.S. (Office of Pesticide Programs, EPA)

Both Agencies have recognized the need to consider sensitivity to chemicals among a broad range of ecologically-relevant plant families. The Agencies have proposed to increase the number of families tested to reduce uncertainty and variability with respect to sensitivity. Researchers have recommend a test battery including non-crop and woody species to encompass a range of response until sufficient comparative toxicity studies are available to determine sensitivity ratios. The selection of the families and the species within those families was based on the feasibility of using them as test species and their economic or ecological importance.

- What are the Panel's thoughts on the proposed terrestrial species at Level 1?
- What (if any) additional species or groups are not adequately represented in the proposed testing scheme at Level 1?
- Are there better approaches for selection of species besides the taxonomic / phylogenetic approach (e.g., ecological or functional approach)? What are they?
- How can the agencies improve their knowledge of the variability in sensitivity of the proposed test species?

Lead Discussants: (selected terrestrial Panel members)

LEVEL 2 and 3 TESTING

Question 13: Additional Species Testing
Michael Davy, B.S. (Office of Pesticide Programs, EPA)

Level 2 is envisioned as primarily an assessment level that utilizes refined exposure methods and toxicity assessment. However, additional testing may be needed to clarify uncertainties before advancing to Level 3, such as laboratory to field extrapolation or specific dose-response curves. Level 3 is envisioned to include expansion of testing in two areas: reproductive testing and acute testing of keystone species. Keystone species can be selected in a couple of ways: (1) keystone species **within families** triggered by risk identified at Level 2; and (2) keystone species within new families that are **within a structure/function group** (e.g., woody plants) identified to be at risk at Level 2 or identified in incident reports.

– What are the Panel's thoughts on additional testing to clarify uncertainties on previously tested species in Level 2?

S What are the Panel's thoughts on having two areas of focus in Level 3 (reproductive testing and acute testing of keystone species)?

S What are the advantages and disadvantages of additional species testing at Level 3? Should the additional species be focused on keystone or ecologically significant species prevalent in areas of chemical use?

S What are the Panel's thoughts on expanded testing of species in sensitive structure/function groups?

Lead Discussants: (selected terrestrial Panel members)

LEVEL 4 TESTING

Question 14: Aquatic and terrestrial multi-species testing

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

Multi-species testing is proposed at the comprehensive level of assessment (Level 4). Population dynamics and community structure could be affected due to differences in chemical sensitivity among individual species. This may result in an alteration of plant community structure which subsequently may lead to adverse effects on organisms at higher trophic levels. Multi-species studies provide necessary and invaluable information about changes in population and community dynamics that result from phytotoxic impacts.

- **S** How useful are data generated from multi-species/community level studies?
- **S** When is multi-species testing appropriate in the proposed design (i.e., how should it be triggered)?
- **S** How many trophic levels should be considered in a multi-species test when considering the risk of chemicals to plants?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

Question 15: Aquatic and terrestrial post-registration monitoring

Michael Davy, B.S. (Office of Pesticide Programs, EPA)

Post-registration monitoring is proposed at the comprehensive level of assessment (Level 4) when adverse effects are anticipated for sensitive species or groups (identified at Level 3). The location and number of monitoring studies will depend on the sensitive species or groups identified and on the types of eco-regions in which they occur. A monitoring study can focus on an indicator species expected to be sensitive, or a multi-species testing design can be introduced to consider the effects on the whole community.

- **S** What are the advantages and disadvantages of monitoring studies focused on indicators versus multi-species (communities)?
- **S** What criteria are used in the selection of an indicator plant species?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

SPECIALIZED TESTING

Question 16: Bioaccumulation

Derek François, M.Sc. (Pest Management Regulatory Agency, Health Canada)

There is a potential for bioaccumulation of chemicals in non-target plants. Bioaccumulation may be one indicator of hazard and more importantly an indication of the extent of uptake and translocation of chemicals in plants. Chemicals that bioaccumulate in plants may also have implications for herbivorous wildlife species. The Agencies are less certain on whether to assess the effects of bioaccumulation in the determination of overall risk to non-target plants.

- **S** What are the SAP's thoughts on the need for uptake / accumulation tests to address bioaccumulation in plants?

- **S** How should the agencies address bioaccumulation?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)

RECOMMENDATIONS

Question 17: Research

Michael Davy, B.S. (Office of Pesticide Programs, EPA)

Since the last SAP meeting, ORD has developed test methods, including *Lemna* and *Arabadopsis* life cycle tests. ORD has also conducted comparative toxicity laboratory and field studies for herbicide effects on annual and woody plants. In addition, ORD has studied short- and long-range transport of chemicals, such as ozone and acid rain, and potential impacts of their deposition on sensitive plants, including endangered and forestry species. A long-range transport model was developed by EPA/Duluth and has been used to model atrazine herbicide transport.

- What are the most important short-term (5 years) and long-term (10 years) research initiatives that will improve plant toxicity testing for the regulation of chemicals?

Lead Discussants: (selected Panel members, preferably one aquatic and one terrestrial)